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*Current Clinical Pharmacology*, 2018, *13*, 120-127

RESEARCH ARTICLE

# Analysis of Toxicity Effects of Buspirone, Cetirizine and Olanzapine on Human Blood Lymphocytes: *In Vitro* Model

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**Abstract:** ***Background*:** The current study investigates the cytotoxicity mechanism of common drugs with piperazine ring such as cetirizine, olanzapine and buspirone on human lymphocytes.

***Methods*:** The viability of lymphocytes, reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP) collapse, lysosomal integrity, content of glutathione and lipid peroxidation was determined.

***Results*:** Buspirone and cetirizine showed more toxicity than olanzapine on human lymphocytes with an IC50 value of 200 µg/ml, after 6 h of incubation. Significant ROS formation, MMP collapse, lipid peroxidation, lysosomal damage and elevation of glutathione disulfide (GSSG) were observed in treated lymphocytes concentrations (4, 20, 40 µg/ml) of buspirone and cetirizine.

***Conclusion*:** Our results show the exposure of human lymphocytes with buspirone and cetirizine, which usually happens during the poisoning, triggers oxidative stress and organelle damages. Our study suggests that using antioxidants, mitochondrial and lysosomal protective agents can protect blood lymphocytes, from probable side effects of these highly consumed medications.

ARTICLE HISTORY

Received: September 17, 2017
Revised: March 26, 2018
Accepted: May 01, 2018

*DOI:*
*10.2174/1574884713666180516112920*

**Keywords:** Cetirizine, olanzapine, buspirone, toxicity, mitochondria, lysosome.

## 1. INTRODUCTION

Up to now, only about 27 million compounds have been recorded [1]. Among these chemical agents, drugs are a big family [2]. The physical, chemical and toxicological properties of this family are all related to the unique molecular structure [3].

Drugs with piperazine ring appeared in the drug market for pharmacological purposes. Many drugs contain a piperazine ring such as antihistamines, antidepressants, antipsychotics and *etc*. [4]. A large number of piperazine compounds have antihistamine effects. One of these drugs is cetirizine which inhibits H1 receptors and was used for the treatment of hay fever, allergies, angioedema, and urticaria. Cetirizine is now available over-the-counter (OTC) in many countries. Cetirizine was the highest-grossing new non-food product of 2008 in the United States (US). It is also available as a generic drug in Turkey, Iran, Australia, New Zealand and other countries [5]. Moreover, antipsychotic drugs such as olanzapine also contain a piperazine ring. Olanzapine is an atypical antipsychotic drug and is approved for treatment of schizophrenia and bipolar disorder. The first-line psychiatric treatment for schizophrenia is antipsychotic medications which include olanzapine. There are several studies which have shown that olanzapine and its metabolites are toxic to normal cells [6, 7]. Another group of piperazine drugs is antidepressants. Buspirone belongs to this class. It is primarily used to treat generalized anxiety disorder (GAD). Cytotoxic effects of buspirone were reported [8].

In humans, piperazine derivatives are readily absorbed from the gastrointestinal tract [9]. Therapeutic blood concentrations measured at clinical studies reached approximately 0.02-0.3, 0.001-0.004, and 0.02-0.03 µg/ml for cetirizine, buspirone and olanzapine, respectively. On the other hand, toxic or lethal blood concentrations were reported for cetirizine, buspirone and olanzapine are 2-5, 2-3, and 1 µg/ml, respectively [10].

The immunotoxicity effects of drugs in lymphocytes, as an important cell in defense against foreign agents, result in infectious complications and virus-induced malignancies. A review of drug-induced immunotoxic effects demonstrates that immunotoxicity is a significant cause of morbidity and even mortality [11]. Our aim in this study was to evaluate toxic effects of piperazine-containing drugs including cetiriz-

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2212-3938/18 $58.00+.00 © 2018 Bentham Science Publishers

Current Clinical Pharmacology

ine, buspirone and olanzapine by a cell-based method, to predict probable toxicity of these drugs in the severe overdose conditions.

## 2. MATERIALS AND METHODS

### 2.1. Chemistry

Trypan blue, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), Rhodamine123, bovine serum albumin (BSA), N-(2-hydroxyethyl) piperazine-N′-(2-ethane sulfonic acid) (HEPES), RPMI1640 and FBS (Fetal Bovine serum) were purchased from Gibco, Life Technologies, Grand Island, NY. Ficoll-paque PLUS was obtained from Ge Healthcare Bio-Science. Cetirizine, buspirone and olanzapine, and all other chemicals with purity 99.9% were purchased from Sigma-Aldrich Corp. (Oakville, ON, Canada).

### 2.2. Blood Samples

Blood samples (n=10) were obtained and approved by Blood Administration Center (Tehran, Iran). The studies were done at the School of Pharmacy, Shahid Beheshti University of Medical Sciences. All healthy individuals signed an informed consent form and this work was approved by the Shahid Beheshti University of Medical Sciences research ethics committee [12].

### 2.3. Lymphocytes Isolation

Lymphocytes were isolated from 10 healthy, non-smoking volunteers at age range 25 to 35 years old, who showed no signs of infectious disease symptoms at the time sampling. Lymphocytes were isolated using Ficoll Paque Plus according to the manufacturer's instructions. After isolation, lymphocytes were suspended in RPMI1640 medium with 10 % FBS and L-glutamine. The final lymphocytes concentration in the experiments was $1 \times 10^6$ cells/ml. The viability of the lymphocytes was over 95 % [12].

### 2.4. Lymphocytes Treatment

Piperazine drugs were dissolved in DMSO with 0.05 % concentration. The concentrations of drugs were in the range of 4-400 μg/ml. The lymphocytes were incubated with compounds for 6 h for analyzing of cell viability. Mechanistic oxidative parameters were searched within 3 h. The incubation was done at 37 °C in 5 % $CO_2$ atmosphere [12].

### 2.5. Cell Viability

$1\times10^4$ cells/well were incubated in 96 well plates with and without piperazine drugs for 6 h. At the end of the test 25 μl of MTT (5 mg/ml in RPMI) was added to each well and incubated for 1 h at 37 °C. MTT formazan crystals were dissolved in 100 μl of DMSO, and the absorbance was assessed at 570 nm with ELISA reader. Each group was tested in three different experimental runs with three replicates for each sample [13].

### 2.6. Determination of ROS

To determine the rate of ROS generation induced by piperazine drugs in lymphocytes, dichlorofluorescein diacetate (DCFH-DA) with 1.6 μM concentration was added to the cells. This reagent penetrates into the cell and becomes hydrolyzed to non-fluorescent dichlorofluorescein (DCFH) then it reacts with ROS to form the highly fluorescent dichlorofluorescein (DCF). Cyclosporine A (Cs.A) as an MPT inhibitor and butylated hydroxyl toluene (BHT), an antioxidant for more mechanistic approach, were used. The fluorescence intensity of DCF was assessed using a Shimadzu RF5000U fluorescence spectrophotometer. Excitation and emission wavelengths were 500 nm and 520 nm, respectively. The results were presented here as fluorescent intensity per $10^6$ cells [14, 15].

### 2.7. MMP Assay

Mitochondria uptake the cationic fluorescent dye of rhodamine 123 (1.5 μM), and this reagent has been used for the assessment of mitochondrial membrane potential. The value of rhodamine 123 in the incubation medium was detected using a Shimadzu RF5000U fluorescence spectrophotometer set at 490 nm excitation and 520 nm emission wavelengths. The capacity of mitochondria to take up rhodamine 123 was calculated as a difference in rhodamine 123 fluorescence [14]. Also, Cyclosporine A (Cs.A) as an MPT inhibitor and butylated hydroxyl toluene (BHT), an antioxidant for more mechanistic approach, were used.

### 2.8. Lysosomal Membrane Integrity Assay

Lymphocytes lysosomal membrane integrity was measured with the redistribution of the acridine orange as a fluorescent dye for lysosomal damages. Cell suspension (0.5 mL) that was previously dyed with acridine orange (5 μM) was separated from the incubation medium by centrifuge (1 1000 g× 1 min). Lymphocytes washing was performed twice to remove the fluorescent dye. Cyclosporine A (Cs.A) as an MPT inhibitor and butylated hydroxyl toluene (BHT), an antioxidant for more mechanistic approach, were used. Acridine orange redistribution in the lymphocyte suspension was then determined fluorometrically using a Shimadzu RF5000U fluorescence spectrophotometer set at 495 nm excitation and 530 nm emission wavelengths [15].

### 2.9. Lipid Peroxidation

For measurement of lipid peroxidation in treated cell, this test was conducted by determining the amount of malondialdehyde (MDA) formed during the decomposition of lipid hydroperoxides by following the absorbance at 532 nm in a Beckman DU-7 spectrophotometer [16]. Also, Cyclosporine A (Cs.A) as an MPT inhibitor and butylated hydroxyl toluene (BHT), an antioxidant for more mechanistic approach, were used.

### 2.10. Glutathione (GSH) and Glutathione Disulfide (GSSG)

GSH and GSSG were measured according to the spectrofluorometric method. Each sample was measured in quartz cuvettes using a fluorimeter set at 350 nm excitation and 420 nm emission wavelengths [16].

### 2.11. Statistical Analysis

Data were presented as mean ± SD. Assays were done in triplicate and the mean was used for statistical analysis. Sta-

tistical significance was determined using the one-way ANOVA test, followed by the post-hoc Tukey test when appropriate. Statistical significance was set at $P < 0.05$.

## 3. RESULTS

### 3.1. Cell Viability

Drugs were tested on lymphocytes and indicated a significant decrease in cell viability. Among them, buspirone showed maximum cytotoxic activity. The toxic effect is presumed to be due to the bulkier size. The order of cytotoxicity of these drugs measured by MTT assay is buspirone > cetirizine > olanzapine, Fig. (**2A**-**C**).

### 3.2. ROS Formation

As shown in Fig. (**3**), treatment of the lymphocytes with cetirizine and buspirone at concentrations (4, 20, 40 µg/ml) which are much less than their $IC_{50}$ resulted in significant ($P<0.05$) increased ROS formation compared to the control lymphocytes, but for olanzapine ROS formation observed at the last time interval (3 h) only by the highest-used concentration (40 µg/ml). These changes were in accordance with the order of cytotoxic activity of the tested drugs buspirone > cetirizine > olanzapine (Fig. **3**).

### 3.3. MMP Collapse

For measurement of the mitochondrial membrane potential, mitochondria were dyed with rhodamine 123. Incubation of lymphocytes with 4, 20 and 40 µg/ml of cetirizine and buspirone for 3 hours led to a mitochondrial membrane potential collapse compared to untreated control lymphocytes. Again, for olanzapine, mitochondrial collapse was observed only at the highest concentration (40 µg/ml) (Fig. **4**). These results were also in accordance with the order of ROS formation and cytotoxicity recorded for these dugs.

### 3.4. Lysosomal Integrity

As shown in Fig. (**5**), there was a loss of lysosomal integrity expressed by a decrease in the uptake of acridine orange when lymphocytes were exposed to cetirizine and buspirone

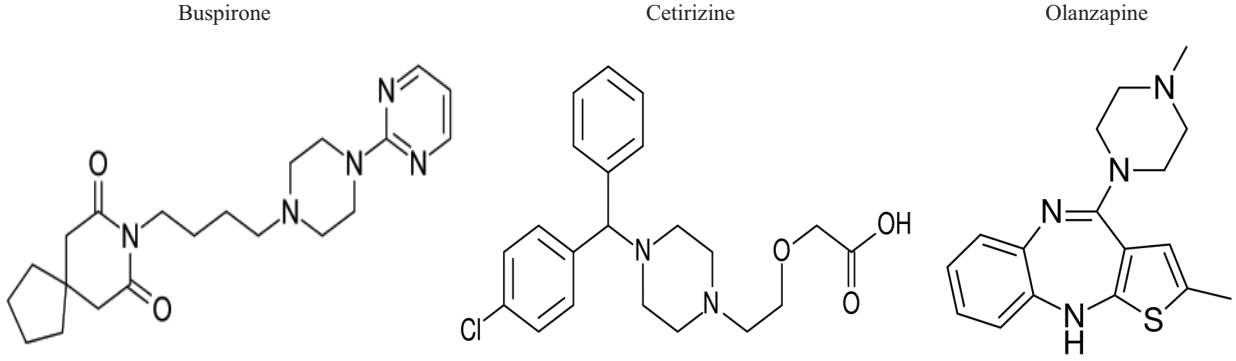


**Fig. (1).** Chemical structure of drugs, Cetirizine (**A**), buspirone (**B**) and olanzapine (**C**).

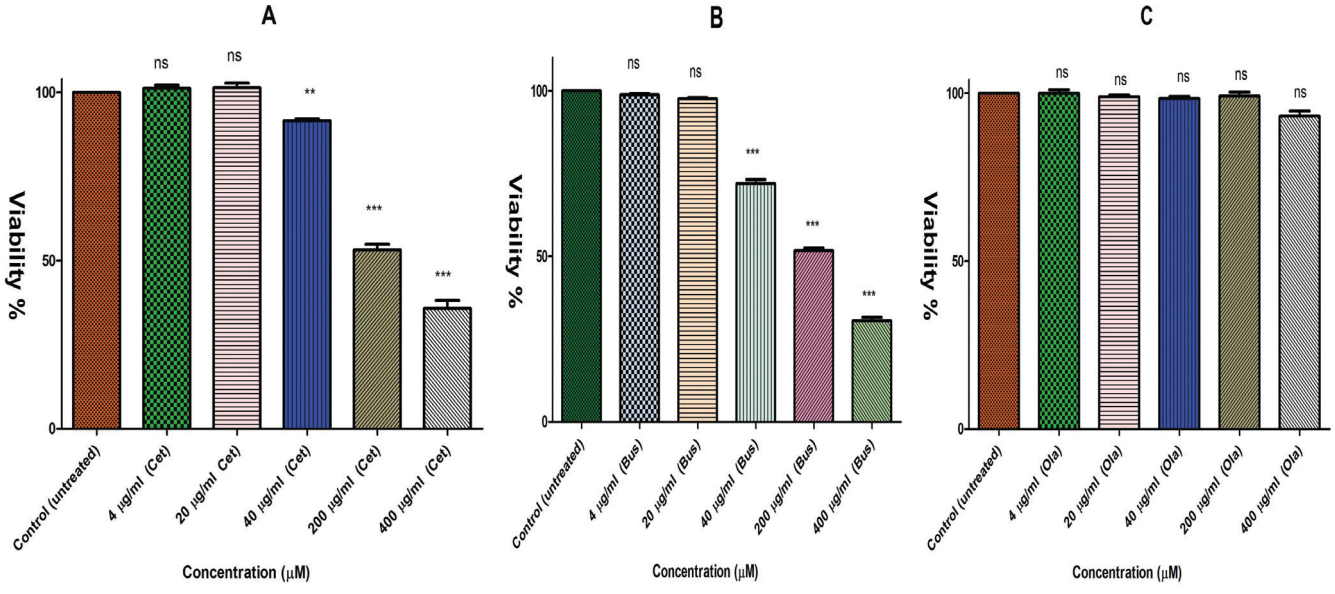


**Fig. (2).** Cytotoxicity, lymphocyte from normal donors, at $1 \times 10^4$ cells/well, was seeded on 96-well plates. Cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at 4-400 µg/ml concentrations were incubated for 6 h. The absorbance representing the viability of lymphocyte was determined by the ELISA reader at 570 nm. Data presented as mean ± SD. The significant level was $P <0.05$ n =5. ** And *** indicate significant ($P <0. 01$) and ($P <0.001$) with untreated control respectively. ns indicates non-significant with control.

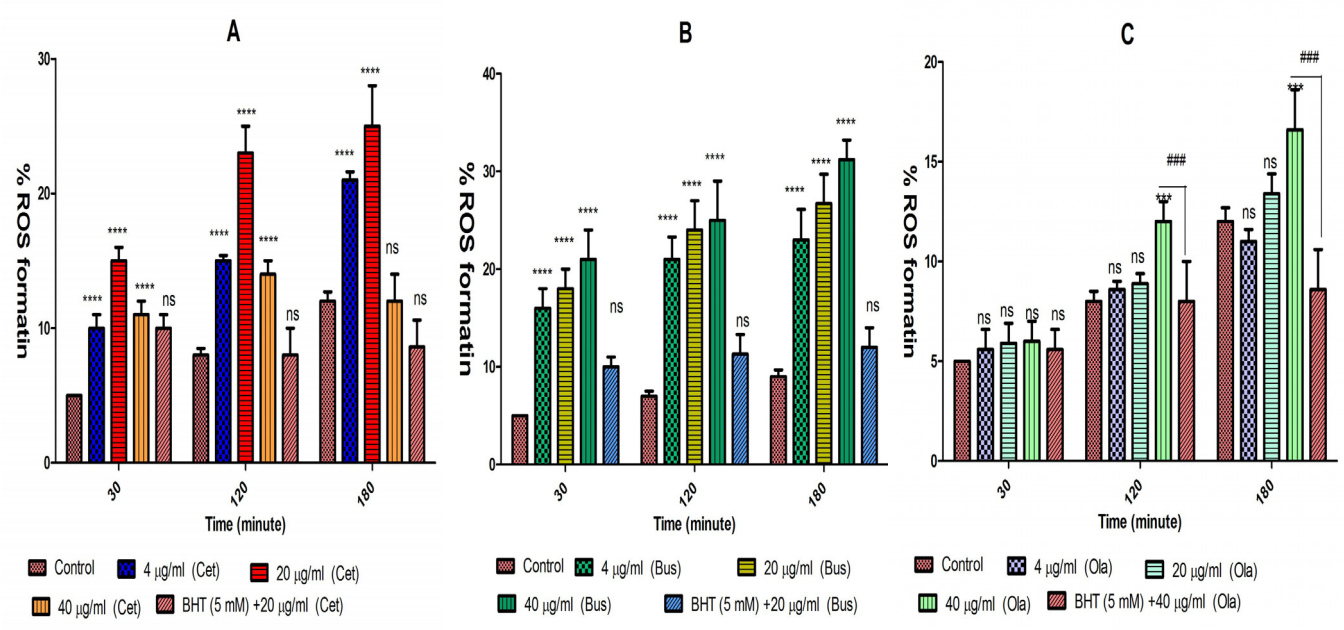


**Fig. (3).** ROS measurement in lymphocytes after exposure with cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at 4, 20 and 40 concentrations within 3h. ROS formation was determined fluorometrically using DCF-DA as described in materials and methods. Values are presented as percent (n = 5). *** represents P < 0.001 significant difference compared to untreated control. ### represents P < 0.001 significant difference compared to 20 µg/ml for each compound. ns indicates non-significant with control.

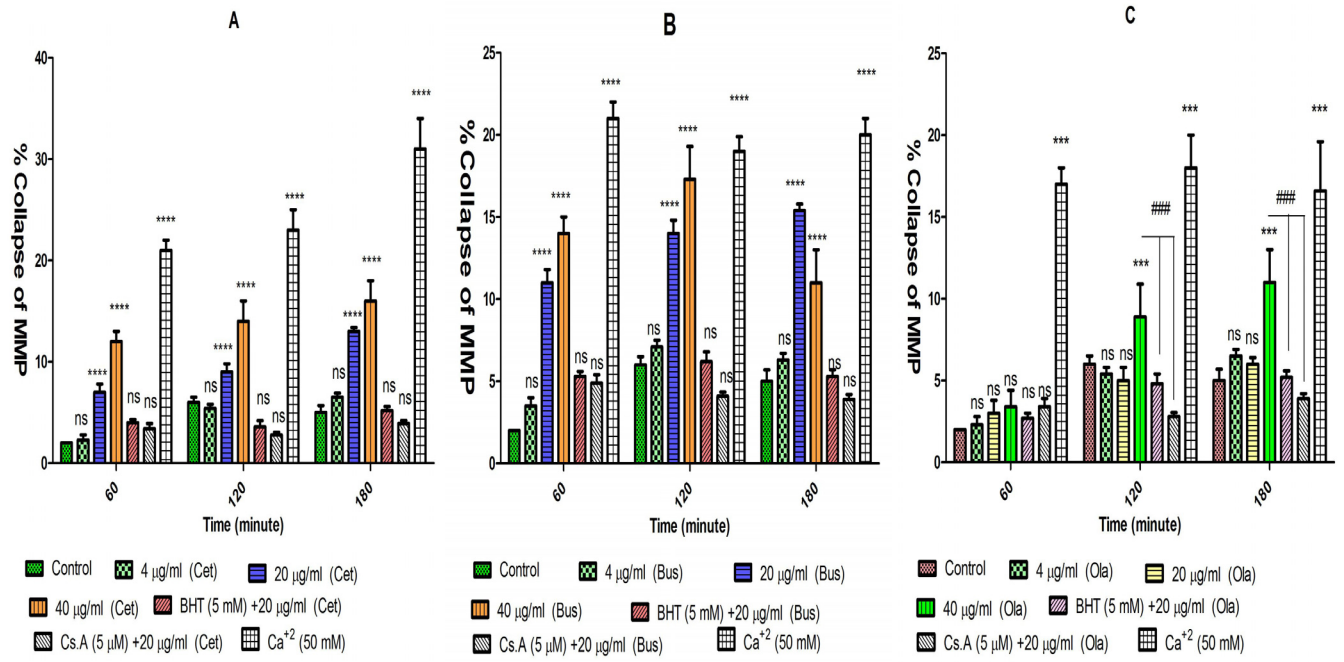


**Fig. (4).** MMP decline in lymphocytes after treatment with cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at 4, 20 and 40 µg/ml concentrations within 3h. Drug-treated compared to those of untreated control. Collapse of MMP was measured by rhodamine 123. Butylated hydroxytoluene (BHT) and MPT pore sealing agents Cyclosporine, A (Cs. A) inhibited the decline of MMP in lymphocytes exposed to 20 or 40 µg/ml for each compound. Values represented as percent of MMP decline (n = 5). *** Represents significant difference ($P$< 0. 001) compared to control. ### Represents significant difference ($P$< 0.001) compared to 20 or 40 µg/ml of each compound. ns indicates non-significant with control.

at concentrations (4, 20 and 40 µg/ml) within 3 h. The loss of lysosomal integrity occurred in a concentration-dependent manner for both drugs within 3 h. Just in case which lymphocytes were incubated with olanzapine for 3h, significant lysosomal damage was occurred only at a concentration of 40 µg/ml.

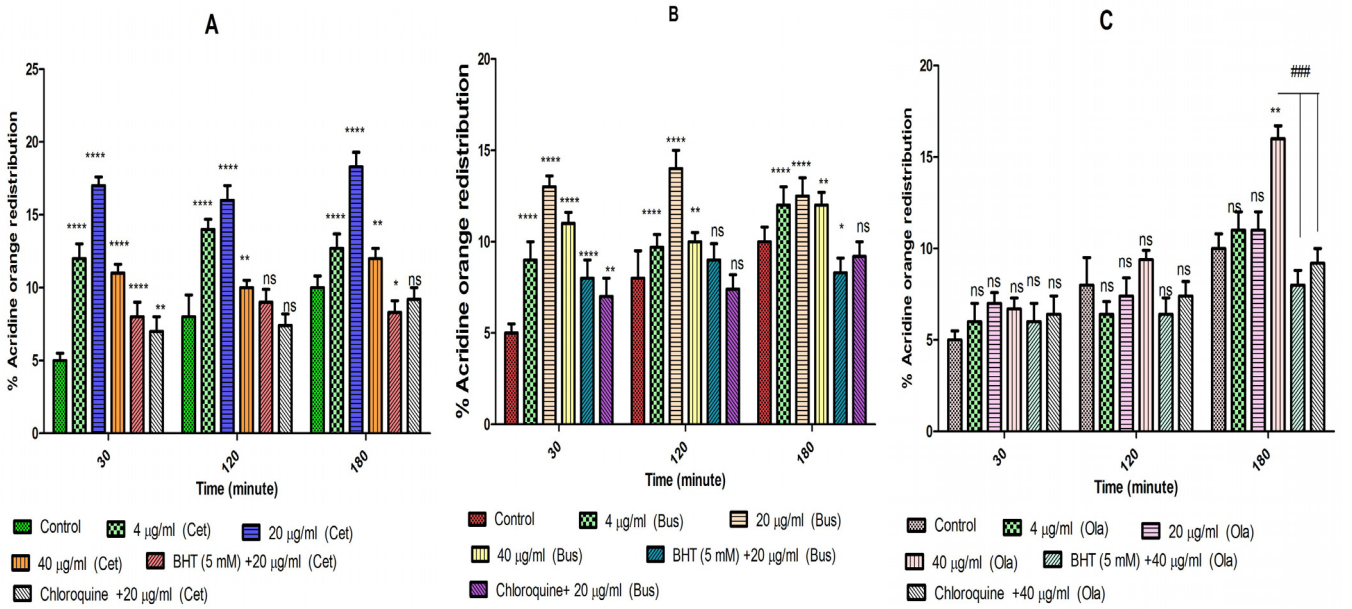


**Fig. (5).** Lysosomal membrane damage after treatment with cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at lymphocytes at 4, 20 and 40 µg/ml concentrations within 3h. Lysosomal membrane damage was measured as a described in materials and methods. Results were shown as % lysosomal membrane leakiness in all treated groups. Values are expressed as mean ± SD of three separate experiments (n = 5). *** Significant difference in comparison with drug treated lymphocytes with control ($p < 0.001$). ns indicates non-significant with control. ### Represents significant difference ($P< 0.001$) compared to 20 or 40 µg/ml of each compound.

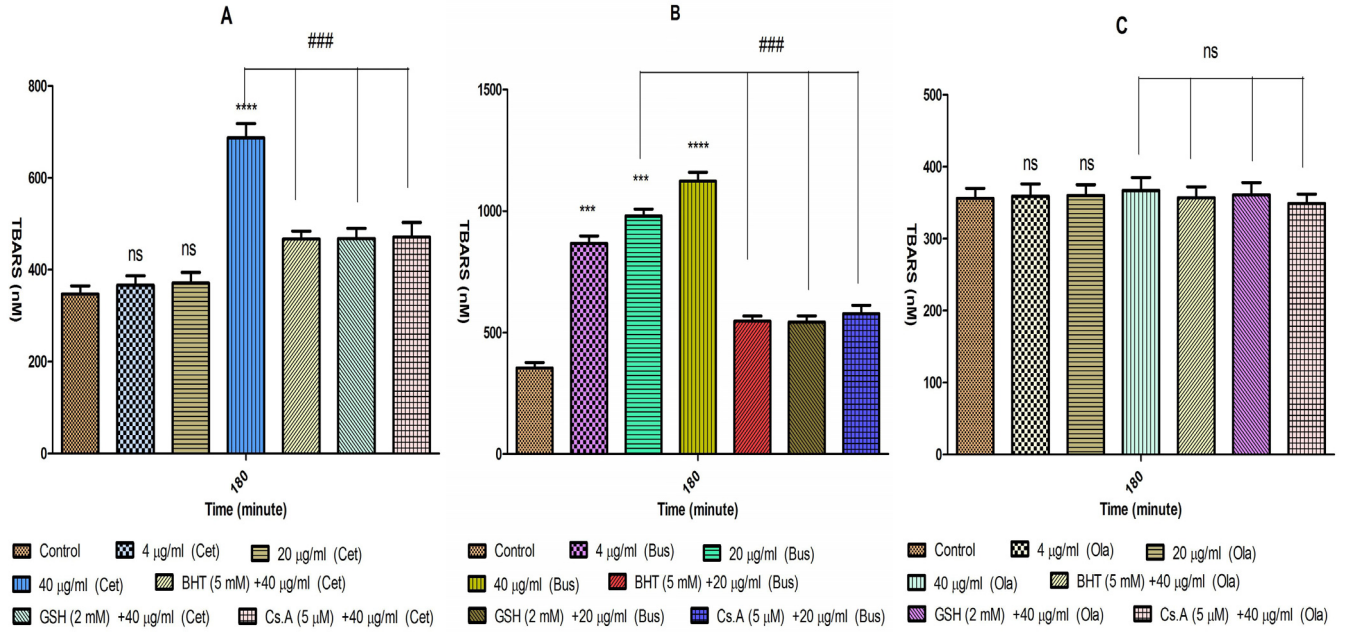


**Fig. (6).** Lipid peroxidation after treatment with cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at lymphocytes at 4, 20 and 40 µg/ml concentrations within 3h. Lymphocytes ($10^6$ cells/ml) were incubated in the RPMI 1640 at 37 $^oC$ for 3 h after the addition of drugs. TBARS formation was presented as nM concentrations. Values are presented as mean ± SD of three separate experiments (n = 5). *** Significant difference in comparison with untreated control lymphocytes ($p < 0.001$). ### Significant difference in comparison with drug-treated lymphocytes (20 or 40 µg/ml) and preventive agent (BHT, GSH and Cs. A) ($p < 0.001$).

### 3.5. Lipid Peroxidation

The ability of the above-mentioned drugs to cause lipid peroxidation in lymphocytes was measured by measuring MDA formation. As shown in Fig. (**6**), buspirone caused significant ($P<0.05$) MDA formation during the 3h incubation at all concentrations (4, 20 and 40 µg/ml) used. But for cetirizine and olanzapine, significant ($P<0.05$) MDA formation could only be observed when lymphocytes were treated with the highest concentration (40 µg/ml).

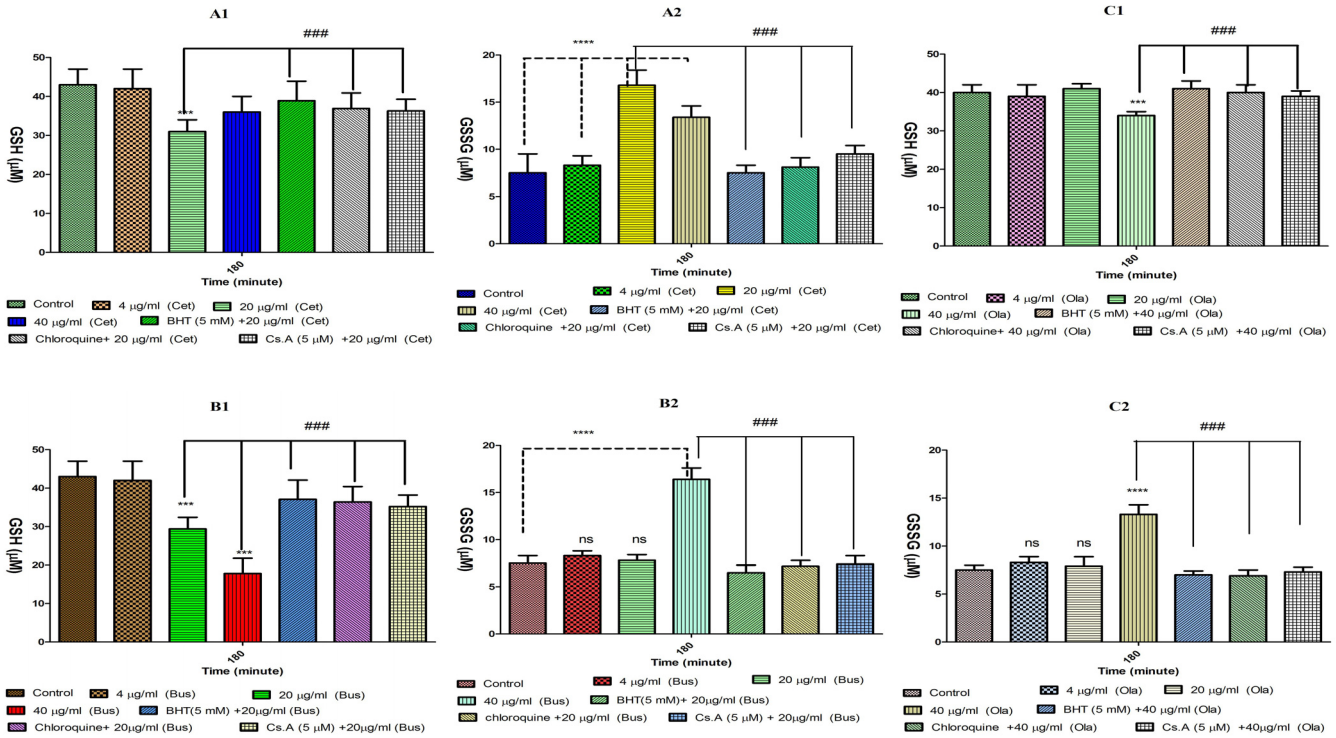


**Fig. (7).** Intracellular GSH (**A1**, **B1** and **C1**) and extracellular GSSG ((**A2**, **B2** and **C2**) after treatment with cetirizine (**A**), buspirone (**B**) and olanzapine (**C**) at lymphocytes at 4, 20 and 40 μg/ml concentrations within 3h. Lymphocytes ($10^6$ cells/ml) were incubated in the RPMI 1640 at 37°C for 3 h after the addition of drugs. Intracellular GSH (**A1**, **B1** and **C1**) and extra cellular GSSG (**A2**, **B2** and **C2**) were fluorimetrically measured. BHT and Cs. A inhibited the decline of GSH in lymphocytes exposed to 20 or 40 μg/ml for each compound. Values are expressed as mean ± SD of three separate experiments (n = 5). *** Significant difference in comparison with control ($p < 0.001$). ns indicates non-significant with control. ### Represents significant difference ($P< 0.001$) compared to 20 or 40 μg/ml of each compound. ns indicates non-significant with control.

### 3.6. Glutathione Levels

As shown in Fig. (**7**), GSH and GSSG levels were measured under the same experimental condition and correlated with MDA formation and other measured parameters. A significant decrease in GSH level was indicated when lymphocytes were treated with 20 and 40 μg/ml of buspirone and cetirizine within 3h which was consequent by an increase in GSSG levels in the treated lymphocytes. There was no significant change in GSH and GSSG levels when lymphocytes were exposed to olanzapine under the same condition.

## 4. DISCUSSION

The properties of a compound such as physical, chemical and toxicological properties are all derived from and related to the unique molecular structure of that compound. This principle forms the basis for the prediction of toxicity from chemical structure [3]. The biological activity of a chemical agent depends on two factors. First, its transformation from the site of administration to the site of action; then its binding or reaction with a receptor or target [1]. In this paper, we studied the cytotoxicity of three drugs with piperazine ring on human blood lymphocytes. A recent survey found that cetirizine, buspirone and olanzapine are amongst the most common drugs with piperazine ring [17]. Our findings showed that all these drugs caused cytotoxicity on human lymphocytes, among them buspirone was the most potent drug to elicit cytotoxic effects.

Oxidative stress is a well-described mechanism which plays an essential role in the cytotoxic effects of several piperazine derivatives that induce the formation of highly reactive species [17]. Interestingly, we found that cetirizine and buspirone significantly increase reactive oxygen species (ROS) formation, as measured through the DCFH-DA assay (Fig. **3**).

Our data also revealed that cetirizine, buspirone and olanzapine alter the mitochondrial depolarization, leading to MMP collapse, which was interestingly inhibited by pretreatment of antioxidant (Butylated hydroxytoluene or BHT) and MPT pore-sealing agents (Cyclosporine, A or Cs.A). After mitochondrial depolarization in the cell, the ATP synthase returns to ATPase activity, consuming ATP and pumping protons outward, in a futile energy-consuming cycle [18]. Other studies conducted with buspirone and olanzapine similarly have demonstrated an MMP collapse ($\Delta\Psi m$) in the cytotoxic effects of these drugs [6, 8]. To confirm that MMP collapse ($\Delta\Psi m$) induced by piperazine drugs results from mitochondrial permeability transition pore(s) (MPTP) opening, we pre-incubated lymphocytes with Cyclosporine A (Cs. A), which binds to cyclophilin D, a part of the MPTP, which causes inhibition of the MPTP opening and prevents

mitochondrial collapse. Our results showed that incubation with Cs.A partially but significantly ($P<0.05$) prevented MMP collapse induced by the tested drugs. Piperazine drug-induced MMP collapse ($\Delta\Psi m$) was also prevented by the ROS scavenger (BHT) suggesting the involvement of increased ROS formation in mitochondrial permeability transition pore(s) (MPTP) opening.

When the loaded lymphocytes were treated with cetirizine and buspirone, indicating leakiness of the lysosomal membrane (Fig. **5**). On the other hand, this drug-induced lysosomal membrane leakiness was prevented by the ROS scavenger (BHT) and lysosomal pH enhancer, Chloroquine. It is assumed that oxidative stress damages both mitochondrial and lysosomal membranes following piperazine drug-induced ROS formation, which leads to cell death.

GSH has an important protective role in oxidant neutralization. Changes in the intracellular amounts of GSH and GSSG are, therefore, powerful indexes of redox disturbances [19]. In agreement with ROS formation data, the intracellular GSH level significantly changed upon incubation with buspirone and cetirizine with the exception of olanzapine. Our data showed a decrease in the intracellular GSH content, accompanied by a significant increase in GSSG levels (Fig. **7**).

It was suggested that lipid peroxidation could have a main role in the toxicity induced by some drugs [20]. The results of the present study also showed that treatment of lymphocytes with buspirone and cetirizine provoked lipid peroxidation in plasma membrane as well as sub-cellular organelle membranes of lymphocytes. Our results suggest that buspirone compared to other piperazine drugs acts as a much stronger pro-oxidant in human blood lymphocytes even at sub-toxic concentrations.

## CONCLUSION

In conclusion, this research for the first time studied the cytotoxicity of cetirizine, buspirone and olanzapine in an ex vivo model, human blood lymphocytes at concentrations much less than IC50. Among three tested drugs, buspirone was the most potent at increasing cytotoxicity parameters. All cetirizine, buspirone and olanzapine induced oxidative stress in human lymphocytes through ROS formation, leading to mitochondrial and lysosomal damages, and GSH depletion. It should be noted that these drugs are frequently consumed in many diseases. We can now conclude that sub-cellular organelle damages and oxidative stress are involved in the toxic effects of these drugs in high overdosed or accumulated concentrations.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work was approved by the Shahid Beheshti University of Medical Sciences research ethics committee.

## HUMAN AND ANIMAL RIGHTS

No Animals were used in the study. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

## CONSENT FOR PUBLICATION

All healthy individuals signed an informed consent form.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

The data provided in this article was extracted from the Pharm D. thesis of Mosleh Razian. The thesis was conducted under the supervision of Prof. Jalal Pourahmad at the Department of Toxicology and Pharmacology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. This study was supported by Shahid Beheshti University of Medical Sciences, Deputy of Research.

## REFERENCES

[1] Dobson CM. Chemical space and biology. Nature 2004; 432(7019): 824-8.

[2] Li JW-H, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009; 325(5937): 161-5.

[3] Barratt MD. Prediction of toxicity from chemical structure. Cell Biol Toxicol 2000; 16(1): 1-13.

[4] Asif M. Piperazine and Pyrazine containing molecules and their diverse pharmacological activities. Int J Adv Scientific Res 2015; 1(1): 5-11.

[5] Portnoy JM, Dinakar C. Review of cetirizine hydrochloride for the treatment of allergic disorders. Expert Opin Pharmacother 2004; 5(1): 125-35.

[6] Eftekhari A, Azarmi Y, Parvizpur A, Eghbal MA. Involvement of oxidative stress and mitochondrial/lysosomal cross-talk in olanzapine cytotoxicity in freshly isolated rat hepatocytes. Xenobiotica 2016; 46(4): 369-78.

[7] Gardner I, Zahid N, MacCrimmon D, Uetrecht JP. A comparison of the oxidation of clozapine and olanzapine to reactive metabolites and the toxicity of these metabolites to human leukocytes. Mol Pharmacol 1998; 53(6): 991-8.

[8] Dykens JA, Jamieson JD, Marroquin LD, *et al.* In vitro assessment of mitochondrial dysfunction and cytotoxicity of nefazodone, trazodone, and buspirone. Toxicol Sci 2008; 103(2): 335-45.

[9] Schep LJ, Slaughter RJ, Vale JA, Beasley DMG, Gee P. The clinical toxicology of the designer "party pills" benzylpiperazine and trifluoromethylphenylpiperazine. Clin Toxicol (Phila) 2011; 49(3): 131-41.

[10] Schulz M, Schmoldt A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. Pharmazie 2003; 58(7): 447-74.

[11] Descotes J. Importance of immunotoxicity in safety assessment: a medical toxicologist's perspective. Toxicol Lett 2004; 149(1-3): 103-8.

[12] Salimi A, Vaghar-Moussavi M, Seydi E, Pourahmad J. Toxicity of methyl tertiary-butyl ether on human blood lymphocytes. Environ Sci Pollut Res Int 2016; 23(9): 8556-64.

[13] Salimi A, Roudkenar MH, Sadeghi L, *et al.* Ellagic acid, a polyphenolic compound, selectively induces ROS-mediated apoptosis in cancerous B-lymphocytes of CLL patients by directly targeting mitochondria. Redox Biol 2015; 6: 461-71.

[14] Rezaei M, Salimi A, Taghidust M, *et al.* A comparison of toxicity mechanisms of dust storm particles collected in the southwest of Iran on lung and skin using isolated mitochondria. Toxicol Environ Chem 2014; 96(5): 814-30.

[15] Hosseini MJ, Shahraki J, Tafreshian S, Salimi A, Kamalinejad M, Pourahmad J. Protective effects of Sesamum indicum extract against oxidative stress induced by vanadium on isolated rat hepatocytes. Environ Toxicol 2016; 31(8):979-85.

[16] Eskandari MR, Fard JK, Hosseini MJ, Pourahmad J. Glutathione mediated reductive activation and mitochondrial dysfunction play key roles in lithium induced oxidative stress and cytotoxicity in liver. Biometals 2012; 25(5): 863-73.

[17] Arbo MD, Silva R, Barbosa DJ, *et al.* Piperazine designer drugs induce toxicity in cardiomyoblast h9c2 cells through mitochondrial impairment. Toxicol Lett 2014; 229(1): 178-89.

[18] Duchen MR. Mitochondria in health and disease: perspectives on a new mitochondrial biology. Mol Aspects Med 2004; 25(4): 365-451.

[19] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012; 5(1): 9-19.

[20] Begriche K, Massart J, Robin M-A, Borgne-Sanchez A, Fromenty B. Drug-induced toxicity on mitochondria and lipid metabolism: mechanistic diversity and deleterious consequences for the liver. J Hepatol 2011; 54(4): 773-94.